Environmental Health Perspectives
Vol. 93, 91–95, 1991

Molecular Determinants of Metastatic Transformation

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In recent years, experimental systems have developed to analyze genetic and epigenetic regulation of the metastatic phenotype. Numerous studies have uncovered a potent role for transforming oncogenes in metastatic conversion. In addition, it has been shown that oncoprotein products operate in a dose-dependent fashion. The continued expression of oncoproteins is required to induce and regulate metastatic dissemination of tumor cells and, consequently, many of the signal transduction pathways that are controlled by the oncogene products can regulate metastasis. Exogenous growth factors that act through these same pathways also alter metastatic potential. Some primary and immortalized cells can be transformed by oncogenes but remain completely benign and nonmetastatic. Malignant transformation can be achieved in these cells through the cooperative interaction of specific oncogenes or loss of active suppression regulated by recessive genetic determinants. Therefore, it is likely that tumor cells acquire the metastatic phenotype through the cooperative interaction of dominant and recessive genetic alterations. This model is consistent with the correlative data accumulating in studies of human tumor specimens where more malignant carcinomas often contain both activating mutations in oncogenes and either inactivating mutations or loss of tumor-suppressor genes.

Introduction

The dissemination of cancer is a complex process requiring many specialized characteristics. In order for a tumor cell to metastasize, it must have the ability to degrade extracellular matrix structures including basement membranes, which line blood vessels and lymphatics. In addition, the metastatic cell must survive hydrostatic pressure drops in the microvasculature, immune surveillance and, finally, possess the ability to bind to endothelium at the secondary site, extravasate out of the vessel, and form an autonomous lesion (*1–3*).

In the past, the metastatic cell has been viewed as one whose phenotype is extremely unstable. It was thought that through this instability, the large number of phenotypic characteristics could be acquired by a single cell. Thus, the metastatic cascade was viewed as a stochastic process involving a large number of gene products. Highly malignant cells are often aneuploid and possess an unstable karyotype; however, it is difficult to imagine that the large number of properties required for metastasis could be achieved by a single cell if they were truly independent events. The effects of this genomic instability may be limited to relatively few genes that can regulate the metastatic phenotype. In this alternative model, instability facilitates tumor progression or evolution, but the genes are regulatory and not a part of the ultimate metastatic phenotype.

There are numerous experiments that demonstrate that many of these "metastatic properties" can be controlled through the same signal transduction pathways as growth and differentiation signals. For example, platelet-derived growth factor (PDGF) can stimulate protease secretion and motility as well as growth or transformation (*4*). Work over the last 5 years on the potential role of oncogenes in metastatic dissemination has provided evidence for this hypothesis (*5–7*).

In 1985, three groups demonstrated that transformation of NIH 3T3 cells by *ras* oncogenes resulted in cell lines that expressed the full metastatic phenotype in nude mice (*8–10*). This question was being independently examined by several laboratories including our own (*7*) and was confirmed and then extended by demonstrating that introduction of *ras* into many types including primary rat fibroblasts also induced the metastatic phenotype (*11,12*). These experiments raised the question of whether *ras* was directly regulating metastatic behavior or was inducing a change in phenotypic stability that would cause metastatic dissemination in a *ras*-independent manner. In addition, it was not clear if *ras* was inducing the metastatic phenotype through the same mechanism that it could transform these cells.

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Direct Regulation of the Metastatic Phenotype

Temperature-sensitive alleles of oncogenes have been isolated which demonstrated that the transformed phenotype was dependent on the continuous presence of the oncoprotein. Inducible transformation systems have also been established with the mouse mammary tumor virus (MMTV) long terminal repeat (LTR) directing transcription of oncogenes such as v-*ras* (*13*). In this case, the transformed phenotype was induced in a time- and dose-dependent manner through addition of glucocorticoids that initiated transcription of the oncogene. The transformed phenotype was therefore dependent not only on the continuous presence of the oncoprotein but on the level of its expression.

Chambers and Wilson demonstrated that metastatic properties of v-*src*-transformed cells were dependent on the continuous presence of $pp60^{src}$ activity using NRK cells transformed by a temperature-sensitive v-*src* gene in the chicken embryo assay (*14*). Our laboratory has shown a correlation between *ras* gene expression and metastatic potential using *ras*-transformed 10T1/2 fibroblasts. In addition, we found that induction of an MMTV-LTR regulated v-H-*ras* gene in NIH 3T3 cells resulted in increased metastatic potential of these cells (*15*). Thus, the dose-dependent regulation of the transformed phenotype by oncogene products was also observed for the metastatic phenotype.

Are the *ras*-Transformed and Metastatic Phenotypes Genetically Separable?

Muschel et al. transformed NIH 3T3 cells with either normal cellular or viral H-*ras* (*16*). Cells transformed by the viral gene were extremely metastatic, whereas the proto-oncogene transformants did not metastasize (*16*). In contrast, Bradley et al. reported that NIH 3T3 cells transformed by *ras* with one of several different mutations including codons 12, 59, and 61, as well as the overexpressed proto-oncogene, were all able to induce the metastatic phenotype (*17*). These data can be reconciled if the proto-oncogene is less potent at inducing the metastatic phenotype than the oncogene. This difference can be noted in transformation assays and has now been documented in two systems involving metastatic conversion (*18,19*).

Our laboratory has extended the range of transforming *ras* alleles that can induce the metastatic phenotype in NIH 3T3 cells to include guanine nucleotide binding mutants in addition to those genes Bradley et al. studied (*17*). In no case has a *ras* gene been found that can transform NIH 3T3 cells without being able to induce the metastatic phenotype in these cells. Thus far, differences in the ability to induce metastasis has been reflected at least partially in differences in transforming potential. This suggests that the mechanism through which *ras* induces the metastatic phenotype in NIH 3T3 cells is likely the same mechanism by which it transforms these cells. In addition, it has been found that *ras* oncogenes can convert many different cells into metastatic tumors (*7,12*). Although this information does not shed any light on the signaling pathway p21 *ras* uses, it does tell us that in malignant tumors in which *ras* signaling is altered, this pathway may be regulating metastatic potential.

Other Oncogenes Can Induce the Metastatic Phenotype

Although *ras* can induce and regulate the metastatic behavior of many experimental cell systems, it is clear that not all metastatic tumors contain activated *ras* genes. Can other transforming oncogenes also induce the metastatic phenotype? In order to test this question, we analyzed NIH 3T3 cells transformed by serine/threonine kinase oncogenes such as *mos* and *raf* as well as by three different classes of tyrosine kinase oncogenes represented by *fms*, *src*, and *fes*. These genes were also capable of inducing metastatic transformation of NIH 3T3 cells (*20*). In contrast, these cells transformed by *myc* or p53 were completely nonmetastatic. As mentioned above, it had previously been shown that v-*src*-transformed NRK cells expressed metastatic properties in the chicken embryo CAM vein experimental metastasis assay developed by Chambers and co-workers (*14*). In addition, Sadowski et al. reported that CCL39 fibroblasts transformed by v-*fps* were metastatic (*21*), and Gao et al. found that v-*mos* transformation of mink lung cells resulted in expression of the metastatic phenotype (*22*).

The *myc* and p53 genes have also been shown to potently regulate metastasis formation in other systems. Bernards et al. have shown that enhanced expression of N-*myc* in the rat neuroblastoma cell line B104 results in a transition from poorly metastatic to a highly invasive, metastatic, and lethal tumor (*23*). In addition, a mutant p53 gene converted mouse bladder carcinoma cells to a highly metastatic tumor (*24*). Transformation of many cells by a number of structurally different types of signal transducing gene products results in metastatic conversion. Not all oncogenes, however, can or will induce the metastatic phenotype in a particular tumor system. As mentioned previously, *myc*- and p53-transformed NIH 3T3 cells are not metastatic (*20*). In addition, primary rat embryo fibroblasts transformed by the combination of E1A type 2 and *ras* were not metastatic, in contrast to these cells transformed by *ras* alone, which are highly malignant (*11*).

Growth Factor Regulation of the Metastatic Phenotype

Many of the oncogenes encode proteins that are directly involved in growth factor signaling. These include

sis, which is the B-chain of PDGF, *erb*-B, which is the receptor for EGF, and *fms*, which is the receptor for CSF-1 (*25*). Since several oncogenes including the tyrosine kinase receptor *fms* were found to induce the metastatic conversion of NIH 3T3 cells, it would seem likely that signaling through these growth control pathways could normally regulate metastatic potential. We have found that NIH 3T3 cells transformed through the expression of a secreted form of bFGF are highly metastatic. In contrast, transient treatment of *ras*- or *src*-transformed NIH 3T3 cells prior to intravenous injection resulted in a profound inhibition of lung colony or experimental metastasis formation (unpublished data). Therefore, growth factor regulation of metastatic properties can be either stimulatory or inhibitory depending on the context of the signal. It has been previously reported that stimulation of B16 murine melanoma cells by the tumor promoting phorbol ester 12-*O*-tetradecanoylphorbol acetate, which activates protein kinase C, results in a stimulation of experimental metastasis formation after IV injection (*26*). This phenomena also occurs in *ras*-transformed 10T1/2 cells (unpublished data). Consequently, several signal transducing pathways within cells can regulate metastatic potential.

Another class of soluble regulators of metastatic dissemination exists, which, in many cases, may have profound influence on the course of a tumor. Steroid hormones such as estradiol are important mitogens for many tumors. In the case of breast cancer, a major form of therapy involves blocking the estrogen signals that reach tumor tissue. This can be achieved through ovariectomy or through the administration of anti-estrogens. Estrogens have been found to not only influence the mitotic status of the tumor, but work in experimental systems suggests that estrogen actually stimulates dissemination of the tumor (*27*). Clinically important regulators of a tumor can operate through many growth regulating pathways. In some cases, signal transduction may be altered within the tumor cell itself as a result of mutation of a gene product that operates on the pathway, such as a growth factor or steroid receptor. In other cases, a tumor may be receiving signals through the same pathway from a nontumor source such as the stroma with which the tumor is in contact, or from a paracrine or endocrine source elsewhere in the body.

Cooperation between Dominant and Recessive Oncogenes

Metastatic transformation *in vitro* by *ras* is limited to some established cell lines and primary cells only under special conditions. A single genetic lesion is normally insufficient not only for induction of the metastatic phenotype but even for tumorigenic transformation. For many years it has been known that tumor progression *in vivo* is a multistep process that usually occurs over an extended period of time. In 1983 it was shown that transformation of primary cells *in vitro* required the interaction of at least two cooperating oncogenes (*28,29*). The phenotypic response of cells to an oncogene like *ras* therefore, is dependent on the presence of other oncogenic lesions within the cell. In the same way, metastatic conversion of NIH 3T3 cells by numerous transforming oncogenes is likely dependent on genetic and/or epigenetic alterations which led to *in vitro* establishment of this aneuploid cell line. Muschel et al. have reported that *ras* transformation of another established murine cell line C127 does not result in metastatic conversion (*16*). What factors determine whether a particular oncogene can induce metastatic conversion of a cell?

We have previously reported that *ras* transformation of 10T1/2 fibroblasts induces the metastatic phenotype (*15*). In order to learn about the events that facilitate susceptibility to metastatic conversion, these cells were transfected with *myc* or *ras* or the sequential combination of both genes in dialyzed calf serum. Under these conditions neither gene alone is transforming (*30*). The combination of both genes resulted in potent transformation. Cell lines derived in this way were both anchorage independent and tumorigenic. In contrast to 10T1/2 cells transformed by *ras* alone, most *myc*/*ras* transformants were nonmetastatic. The *myc* gene had made 10T1/2 cells highly susceptible to *ras*-mediated transformation but had not fully complemented *ras* in allowing for metastatic conversion.

The *ras* gene alone can induce the full metastatic phenotype when introduced in the presence of a factor present in fetal calf serum (*15,30*). Consequently, an additional event in cooperation with *ras* is necessary for metastatic conversion. Introduction of *myc* into the *ras* metastatic transformants does not suppress the metastatic phenotype, therefore, metastasis is not inhibited by *myc*. Finally, it was found that fusion of the nonmetastatic *myc*/*ras* transformants to the metastatic *ras* transformants resulted in mostly nonmetastatic hybrids, indicating that the metastatic phenotype was recessive. The cooperating event in this system was likely to involve the loss of or inactivation of a recessive determinant. It has also been shown in naturally derived tumor cells that fusion of metastatic and nonmetastatic cells resulted in the nonmetastatic hybrids (*31*). It is likely, therefore, that the metastatic phenotype is achieved through the cooperative interaction of both dominant and recessive determinants.

Summary and Studies on Human Tumors

An independent line of study has helped elucidate events that are likely important in human tumor progression. In the last 3 years, several teams of researchers have uncovered provocative correlations between specific genetic alterations and malignancy in neoplasia of the breast, lung, and colon. Specific alterations have been detected at several loci in human breast cancer. The most striking finding, however, has been a correlation between malignant behavior and amplification/

overexpression of the HER2/neu (c-*erb*-B2) growth factor receptor (*32–34*). This gene product is apparently wild-type but through overexpression renders tumor tissue highly sensitive to the neu ligand (*35*). The studies described above may provide a mechanistic framework for the interpretation of this clinical data. The neu ligand, whether produced by tumor or by nontumor tissue, may be promoting not only growth but dissemination. The picture is likely not universal for all breast cancer.

Many patients with metastatic breast cancer have tumor cells that express low levels of HER2. In these cases, other lesions such as amplifications of the growth factor *int*-2 gene, c-*myc*, and/or alterations of the tumor-suppressor genes p53 and *Rb*, which are often found (*36*), may be important determinants of metastatic potential. In human lung cancer, numerous different genetic alterations have been found within single tumors (*37*). In small cell as well as nonsmall cell carcinomas, mutations have been found frequently in K-*ras*, *Rb*, and p53. In addition, *Rb* and p53 and other loci on chromosomes 3 and 11 are reduced to homozygosity, suggesting that these loci are functioning as recessive determinants of malignancy and that alterations at each of the two alleles separately contribute to the malignant phenotype. In highly metastatic small cell lung cancer, one of the c-, N-, or L-*myc* genes are often amplified. These tumors are usually highly disseminated, resistant to therapy, and ultimately lethal.

Vogelstein and co-workers have recently presented a model for tumor progression of colon carcinoma (*38–42*). In this model, loss of tumor-suppressor genes on chromosomes 5, 17 (p53), and 18 (DCC) cooperate with mutational activation of K-*ras* and p53. The order in which these events occur is variable, but the chromosome 5 and K-*ras* alterations often occur early and prior to frank carcinoma. In contrast, mutations at the p53 and DCC genes often occur at the transition to carcinoma *in situ* or later. Since the *ras* gene activation is often present in benign adenomas, it cannot be responsible for induction of metastasis formation. On the other hand, these mutations have been observed to occur in a different order, suggesting that each alteration may be capable of contributing a progressive effect to tumors at many different stages in the course of tumor evolution. If this is the case, then dissemination of the resulting malignant tumor may be controlled through the cooperative interaction of these and possibly as yet unidentified genetic events. In other words, although one specific genetic lesion may be able to promote transition of a tumor from nonmetastatic to metastatic, the events that preceded this ultimate change are all likely important in the maintenance and regulation of the metastatic phenotype. Thus, the regulation of metastatic dissemination may be dependent on the cooperative interaction of many specific genetic alterations that are present in some malignant tumors. If all or most of the molecular lesions are necessary for its dissemination, this would predict that therapeutic approaches may need to only reverse a single important genetic event.

This work was supported by the National Cancer Institute of Canada. S. E. Egan is an NCIC fellow, A. H. Greenberg is a Terry Fox Cancer Research Scientist, and J. A. Wright is a Senior Research Scientist of the NCIC.

REFERENCES

1. Poste, G., and Fidler, I. J. The pathogenesis of cancer. Nature 282: 139–145 (1980).
2. Nicolson, G. Cancer metastasis: organ colonization and cell-surface properties and malignant cells. Biochim. Biophys. Acta 695: 113–176 (1982).
3. Liotta, L. A. Tumor invasion and metastasis—role of the extracellular matrix: Rhoads memorial award lecture. Cancer Res. 46: 1–7 (1986).
4. Deuel, T. F. Polypeptide growth factors: roles in normal and abnormal cell growth. Annu. Rev. Cell. Biol. 3: 443–492 (1987).
5. Muschel, R., and Liotta, L. A. Role of oncogenes in metastases. Carcinogenesis 9: 705–710 (1988).
6. Steeg, P. S. Search for metastasis suppressor genes. Invasion Metastasis 9: 351–359 (1989).
7. Greenberg, A. H., Egan, S. E., and Wright, J. A. Oncogenes and metastatic progression. Invasion Metastases 9: 360–378 (1989).
8. Thorgeirsson, U. P., Turpeenniemi-Huganen, T., Williams, J. E., Weti, E. H., Heilman, C. A., Talmadge, J. E., and Liotta, L. A. NIH-3T3 cells transfected with human tumor DNA containing *ras* oncogenes express the metastatic phenotype in nude mice. Mol. Cell. Biol. 5: 259–262 (1985).
9. Bernstein, S. C., and Weinberg, R. A. Expression of the metastatic phenotype in cells transfected with human metastatic tumor DNA. Proc. Natl. Acad. Sci. U.S.A. 82: 1726–1730 (1985).
10. Greig, R. G., Koestler, T. P., Trainer, D. L., Corwin, S. P., Miles, L., Kline, T., Sweet, R., Yokoyama, S., and Poste, G. Tumorigenic and metastatic properties of "normal" and *ras*-transfected NIH-3T3 Cells. Proc. Natl. Acad. Sci. U.S.A. 82: 3698–3701 (1985).
11. Pozzatti, R., Muschel, R., Williams, J., Padmanabhan, R., Howard, B., Liotta, L. A., and Khoury, G. Primary rat embryo cells transformed by one or two oncogenes show different metastatic potentials. Science 232: 223–227 (1986).
12. Liotta, L. A. Editorial: H-*ras* p21 and the metastatic phenotype. J. Natl. Cancer Inst. 80: 468–469 (1986).
13. Huang, A. L., Ostrowski, M. C., Berard, D., and Hager, G. L. Glucocorticoid regulation of the Ha-MuSV p21 gene conferred by sequences from the mouse mammary tumor virus. Cell 27: 245–255 (1981).
14. Chambers, A. F., and Wilson, S. Cells transformed with a ts viral *src* mutant are temperature sensitive for *in vivo* growth. Mol. Cell. Biol. 5: 728–733 (1985).
15. Egan, S. E., McClarty, G. A., Jarolim, L., Wright, J. A., Spiro, I., Hager, G., and Greenberg, A. H. Expression of H-*ras* correlates with metastatic potential: evidence for direct regulation of the metastatic phenotype in 10T1/2 and NIH-3T3 cells. Mol. Cell. Biol. 7: 830–837 (1987).
16. Muschel, R. J., Williams, J. E., Lowy, D. R., and Liotta, L. A. Harvey *ras* induction of metastatic potential depends upon oncogene activation and the type of recipient cell. Am. J. Pathol. 121: 1–8 (1985).
17. Bradley, M. O., Kraynak, A. R., Strorer, R. D., and Gibbs, J. B. Experimental metastasis in nude mice of NIH-3T3 cells containing various *ras* genes. Proc. Natl. Acad. Sci. U.S.A. 83: 5277–5281 (1986).
18. Waghorne, C., Kerbel, R. S., and Breitman, M. L. Metastatic potential of SP1 mouse mammary adenocarcinoma cells is differentially induced by activated and normal forms of c-H-*ras*. Oncogene 1: 149–155 (1987).
19. Egan, S. E., Broere, J. J., Jarolim, L., Wright, J. A., and Greenberg, A. H. Co-regulation of metastatic and transforming activity of normal and mutant *ras* genes. Int. J. Cancer 43: 443–449 (1989).
20. Egan, S. E., Wright, J. A., Jarolim, L., Yanagihara, K., Bassin, R. H., and Greenberg, A. H. Transformation by oncogenes en-

coding protein kinases induces the metastatic phenotype. Science 238: 202–205 (1987).
21. Sadowski, I., Pawson, T., and Lagarde, A. v-*fps* protein-tyrosine kinase coordinately enhances the malignancy and growth factor responsiveness of pre-neoplastic lung fibroblasts. Oncogene 2: 241–248 (1988).
22. Gao, C., Wang, L. -C., and Voss, W. C. The role of v-*mos* in transformation, oncogenicity and metastatic potential of mink lung cells. Oncogene 3: 267–273 (1988).
23. Bernards, R., Dessain, S. K., and Weinberg, R. A. N-*myc* amplification causes down-modulation of MHC class 1 antigen expression in neuroblastoma. Cell 47: 667–674 (1986).
24. Pohl, J., Goldfinger, N., Radler-Pohl, A., Rotter, V., and Schirrmacher, V. p53 increases experimental metastatic capacity of murine carcinoma cells. Mol. Cell. Biol. 8: 2078–2081 (1988).
25. Sherr, C., J., Rettenmier, C. W., Sacca, R., Roussel, M. F., Look, A. T., and Stanley, E. R. The c-*fms* proto-oncogene product is related to the receptor for the mononuclear phagocyte growth factor, CSF-1. Cell 41: 665–676 (1985).
26. Gopalakrishna, R., and Barsky, S. H. Tumor promoter-induced membrane-bound protein kinase C regulates hematogenous metastasis. Proc. Natl. Acad. Sci. U.S.A. 85: 612–615 (1988).
27. Albini, A., Graf, J., Kitten, G. T., Kleinmon, H. K., Martin, G. R., Veillette, A., and Lippman, M. E. 17β-estradial regulates and v-H-*ras* transfection constitutively enhances MCF-7 breast cancer cell interactions with basement membrane. Proc. Natl. Acad. Sci. U.S.A. 83: 8182–8186 (1986).
28. Land, H., Parada, L. F., and Weinberg, R. A. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. Nature 304: 596–602 (1983).
29. Ruley, E. H. Adenovirus early region 1A enables viral and cellular transforming genes to transform primary cells in culture. Nature 304: 602–606 (1983).
30. Hsiao, W. -L. W., Lopez, C. A., Wu, T., and Weinstein, I. B. A factor present in fetal calf serum enhances oncogene-induced transformation of rodent fibroblasts. Mol. Cell. Biol. 7: 3380–3385 (1987).
31. Turprenniemi-Hijanen, T., Thorgeirrson, V. P., and Hart, I. R. Expression of collagenase IV activity in murine tumor cell hybrids that differ in metastatic potential. J. Natl. Cancer Inst. 75: 99–103 (1985).
32. Slamon, D. J., Clark, G. M., Wong, S. G., Levin, W. J., Ullrich, A., and McGuire, W. L. Human breast cancer correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235: 177–182 (1987).
33. Slamon, D. J., Godolphin, W., Jones, L. A., Holt, J. A., Wong, S. G., Keith, D. E., Levin, W. J., Stuart, S. G., Udove, J., Ullrich, A., and Press, M. F. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 244: 707–712 (1989).
34. Marx, J. L. Gene signals relapse of breast, ovarian cancers. Science 244: 654–655 (1989).
35. Yarden, Y., and Weinberg, R. A. Experimental approaches to hypothetical hormones: detection of a candidate ligand of the neu proto-oncogene. Proc. Natl. Acad. Sci. U.S.A. 86: 3179–3183 (1989).
36. Lee, E., To, H., Shew, J. -Y., Bookstein, R., Scully, P., and Lee, W. -H. Inactivation of the retinoblastoma susceptibility gene in human breast cancers. Science 241: 218–221 (1988).
37. Weston, A., Willey, J. C., Modali, R., Sugimura, H., McDowell, E. M., Resau, J., Light, B., Haugen, A., Mann, D. L., Trump, B. F., and Harris, C. C. Differential DNA sequence deletions from chromosomes 3, 11, 13 and 17 in squamous-cells carcinoma, large-cell carcinoma and adenocarcinoma of the human lung. Proc. Natl. Acad. Sci. U.S.A. 86: 5099–5103 (1989).
38. Vogelstein, B., Fearon, E. R., Kern, S. E., Hamilton, S. R., Preisinger, A. C., Nakamura, Y., and White, R. Allelotype of colorectal carcinomas. Science 244: 207–211 (1989).
39. Baker, S. J., Fearon, E. R., Nigro, J. M., Hamilton, S. R., Preisinger, A. C., Jessup, J. M., van Tuinen, P., Ledbetter, D. H., Barker, D. F., Nakamura, Y., White, R., and Vogelstein, B. Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. Science 244: 217–221 (1989).
40. Fearon, E. R., Cho, K. R., Nigro, J. M., Kern, S. E., Simons, J. W., Ruppert, J. M., Hamilton, S. R., Preisinger, A. C., Thomas, G., Kinzler, K. W., and Vogelstein, B. Identification of a chromosome 18q gene that is altered in colorectal cancers. Science 247: 49–56 (1990).
41. Stanbridge, E. J. Identifying tumor suppressor genes in human colorectal cancer. Science 247: 12–13 (1990).
42. Marx, J. Research news: many gene changes found in cancer. Science 246: 1386–1388 (1989).